

Listing of the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1 - 74. (Canceled)

75. (Currently amended) A DNA molecule which encodes an RNA molecule comprising:

- (a) at least one *cis*-acting sequence element,
- (b) a first open reading frame which encodes a non-cytopathic temperature-sensitive ~~alphaviral~~ Sindbis virus replicase, wherein the non-cytopathicity and temperature sensitivity are conferred by one or more mutations in the genes encoding the nonstructural proteins of said replicase is conferred by a substitution of proline at position 726 of the nonstructural protein 2 (nsP2) of the replicase with another naturally occurring amino acid, and
- (c) at least one ~~second~~ nucleotide sequence selected from the group consisting of:
 - (i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;
 - (ii) a sequence complementary to all or part of the second open reading frame of (i); and

- (iii) a sequence encoding an untranslated RNA molecule, or complement thereof;

wherein said ~~second~~ at least one nucleotide sequence of (c) is operably linked to a promoter which is recognized by said non-cytopathic, temperature-sensitive ~~alphaviral~~ Sindbis virus replicase.

76. (Currently amended) The DNA molecule of claim 75, which comprises only one ~~second-nucleotide sequence~~ nucleotide sequence of (c).

77. (Previously presented) The DNA molecule of claim 75, wherein said second open reading frame is in a translatable format after one RNA-dependent RNA replication event.

78. (Previously presented) The DNA molecule of claim 75, wherein said second open reading frame is in a translatable format after three RNA-dependent RNA replication events.

79 - 80. (Canceled)

81. (Canceled)

82. (Previously presented) The DNA molecule of claim 75 which encodes an alphaviral replicase having replicase activity at 34°C which is at least five fold lower than the replicase activity exhibited at 29°C.

83. (Previously presented) The DNA molecule of claim 75, wherein the second open reading frame encodes a cytokine, a lymphokine, a tumor necrosis factor, an interferon, a toxic protein, or a prodrug converting enzyme.

84. (Previously presented) The DNA molecule of claim 75, wherein the second open reading frame encodes human erythropoietin or human β -interferon.

85. (Canceled)

86. (Previously presented) A method of making a recombinant host cell comprising introducing the DNA molecule of claim 75 into a host cell *in vitro*.

87. (Canceled)

88. (Previously presented) An *in vitro* cell culture comprising a recombinant host cell comprising the DNA molecule of claim 75.

89. (Previously presented) The cell culture of claim 88, wherein some or all of the DNA molecule is stably maintained in said host cell.

90. (Currently amended) An RNA molecule ~~transcribed from the DNA molecule of claim 75~~ comprising:

- (a) at least one *cis*-acting sequence element,
- (b) a first open reading frame which encodes a non-cytopathic temperature-sensitive Sindbis virus replicase, wherein the non-cytopathicity is conferred by a substitution of proline at position 726 of the nonstructural protein 2 (nsP2) of the replicase with another naturally occurring amino acid, and
- (c) at least one nucleotide sequence selected from the group consisting of:
 - (i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;
 - (ii) a sequence complementary to all or part of the second open reading frame of (i); and
 - (iii) a sequence encoding an untranslated RNA molecule, or complement thereof;

wherein said at least one nucleotide sequence of (c) is operably linked to a promoter which is recognized by said non-cytopathic, temperature-sensitive Sindbis virus replicase.

91. (Previously presented) An alphaviral particle containing the RNA molecule of claim 90.

92. (Previously presented) An *in vitro* cell culture comprising a recombinant host cell comprising the RNA molecule of claim 90.

93. (Previously presented) A method for producing a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) introducing at least one DNA molecule of claim 75 into said host cells *in vitro*;
- (b) culturing said host cells under conditions suitable for expression of said protein or untranslated RNA molecule; and
- (c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

94. (Previously presented) A method for producing a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) introducing at least one RNA molecule of claim 90 into said host cells *in vitro*;
- (b) culturing said host cells under conditions suitable for expression of said protein or untranslated RNA molecule; and
- (c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

95. (Previously presented) The method of claim 94, wherein the protein is erythropoietin.

96. (Canceled)

97. (Previously presented) A method for producing alphaviral particles, said method comprising:

- (a) introducing into a host cell *in vitro* at least one DNA molecule of claim 75 having one or more open reading frames which encode alphaviral structural proteins;
- (b) growing host cells under culture conditions suitable for the production of alphaviral particles which contain an RNA transcription product of said DNA molecule; and
- (c) recovering said alphaviral particles.

98. (Previously presented) A method for producing a protein encoded by RNA contained in an alphaviral particle produced by the method of claim 97 in a recombinant host cell comprising:

- (a) infecting a host cell *in vitro* with the alphaviral particle;

- (b) growing said host cell under culture conditions suitable for the production of said protein; and
- (c) recovering said protein.

99. (Previously presented) The method of claim 98, wherein said protein is erythropoietin.

100. (Currently amended) A method for regulating the expression of a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) ~~growing host cells under suitable culture conditions~~ introducing at least one DNA molecule of claim 75 into said host cells *in vitro*;
- (b) ~~introducing at least one DNA molecule of claim 75 into said host cells *in vitro*~~ growing host cells under suitable culture conditions; and
- (c) changing the temperature of the host cell culture from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

101. (Currently amended) A method for regulating the expression of a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) ~~growing host cells under suitable culture conditions~~ introducing at least one RNA molecule of claim 90 into host cells *in vitro*;

- (b) ~~introducing at least one RNA molecule of claim 90 into said host cells *in vitro*~~ growing said host cells under suitable culture conditions; and
- (c) changing the temperature of the host cell culture from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said at least one RNA molecule of claim 90.

102. (Previously presented) An isolated nucleic acid molecule comprising a polynucleotide having the nucleotide sequence of SEQ ID NO:1.

103. (Currently amended) A DNA vector system comprising one or more polynucleotides which encode RNA molecules, said RNA molecules comprising:

- (a) at least one *cis*-acting sequence element,
- (b) a first open reading frame having a nucleotide sequence encoding a non-cytopathic, temperature-sensitive ~~alphaviral~~ Sindbis virus replicase, wherein the non-cytopathicity and temperature sensitivity are conferred by one or more mutations in the genes encoding the nonstructural proteins of said replicase is conferred by a substitution of proline at position 726 of the nonstructural protein 2 (nsP2) of the replicase with another naturally occurring amino acid, and

- (c) at least one ~~second~~ nucleotide sequence selected from the group consisting of:
- (i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;
 - (ii) a sequence complementary to all or part of the second open reading frame of (i); and
 - (iii) a sequence encoding an untranslated RNA molecule, or complement thereof;

wherein said ~~second~~ at least one nucleotide sequence of (c) is operably linked to a promoter which is recognized by said non-cytopathic, temperature-sensitive ~~alphaviral~~ Sindbis virus replicase.

104. (Canceled)

105. (Previously presented) The DNA vector system of claim 103 which encodes an alphaviral replicase having replicase activity at 34°C which is at least five fold lower than the replicase activity exhibited at 29°C.

106. (Previously presented) The DNA vector system of claim 103, wherein the second open reading frame encodes a cytokine, a lymphokine, a tumor necrosis factor, an interferon, a toxic protein, or a prodrug converting enzyme.

107. (Previously presented) The DNA vector system of claim 103, wherein the second open reading frame encodes human erythropoietin or human β -interferon.

108. (Canceled)

109. (Currently amended) A method of making a recombinant host cell comprising introducing at least one ~~polynucleotide~~ DNA vector system of claim 103 into a host cell *in vitro*.

110. (Canceled)

111. (Currently amended) An *in vitro* cell culture comprising a recombinant host cell comprising at least one ~~polynucleotide~~ DNA vector system of claim 103.

112. (Previously presented) The cell culture of claim 111, wherein some or all of the polynucleotide sequences of claim 103 are stably maintained in said host cell.

113. (Canceled)

114. (Currently amended) An alphaviral particle containing ~~at least one~~ an RNA molecule of claim ~~443~~ 90.

115. (Currently amended) An *in vitro* cell culture comprising a recombinant host cell comprising at least one RNA molecule of claim ~~113~~ 90.

116. (Currently amended) A method for producing a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) ~~growing host cells under suitable culture conditions~~ introducing at least one DNA vector system of claim 103 into host cells *in vitro*;
- (b) ~~introducing at least one DNA molecule of claim 103 into said host cells *in vitro*;~~ growing said host cells under suitable culture conditions; and
- (c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said at least one nucleotide sequence ~~DNA molecule~~.

117. (Canceled)

118. (Currently amended) The method of claim ~~117~~ 116, wherein ~~the~~ said protein is erythropoietin.

119. (Canceled)

120. (Currently amended) A method for producing an alphaviral particle comprising an RNA encoding a non-cytopathic, temperature-sensitive Sindbis virus replicase, the method comprising:

- (a) ~~growing host cells under suitable culture conditions~~ introducing at least one DNA vector system of claim 103 into host cells *in vitro*;
- (b) ~~introducing into said host cells *in vitro* at least one DNA molecule of claim 103 having one or more open reading frames which encode alphaviral structural proteins~~ growing said host cells under suitable culture conditions;
- (c) producing an alphaviral particle comprising an RNA encoding a non-cytopathic, temperature-sensitive Sindbis virus replicase; and
- (d) recovering said alphaviral particle.

121. (Currently amended) A method for producing a protein in a recombinant host cell comprising:

- (a) ~~growing host cells under suitable culture conditions~~ infecting host cells *in vitro* with an alphaviral particle produced by the method of claim 120, wherein said alphaviral particle comprises an RNA encoding said non-cytopathic, temperature sensitive Sindbis virus replicase;
- (b) ~~infecting said host cells *in vitro* with an alphaviral particle produced by the method of claim 120~~ growing said host cells under suitable culture conditions; and
- (c) recovering said protein;

wherein said protein is encoded by nucleic acid contained in said alphaviral particle.

122. (Previously presented) The method of claim 121, wherein said protein is erythropoietin.

123. (Currently amended) A method for regulating the expression of a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) ~~growing host cells under suitable culture conditions~~ introducing at least one DNA vector system of claim 103 into host cells *in vitro*;
- (b) ~~introducing at least one DNA molecule of claim 103 into said host cells *in vitro*~~ growing said host cells under suitable culture conditions; and
- (c) changing the temperature of the host cell culture from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by ~~said DNA molecule~~ the polynucleotides of said at least one DNA vector system.

124. (Canceled)

125. (Currently amended) A composition comprising one or more RNA molecules, said RNA molecules comprising:

- (a) at least one *cis*-acting sequence element,

- (b) a first open reading frame having a nucleotide sequence encoding a non-cytopathic, temperature-sensitive ~~alphaviral~~ Sindbis virus replicase, wherein ~~the non-cytopathicity and temperature sensitivity are conferred by one or more mutations in the genes encoding the nonstructural proteins of said replicase~~ is conferred by a substitution of proline at position 726 of the nonstructural protein 2 (nsP2) of the replicase with another naturally occurring amino acid, and
- (c) at least one ~~second~~ nucleotide sequence selected from the group consisting of:
 - (i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;
 - (ii) a sequence complementary to all or part of the second open reading frame of (i); and
 - (iii) a sequence encoding an untranslated RNA molecule, or complement thereof;

wherein said ~~second~~ at least one nucleotide sequence of (c) is operably linked to a promoter which is activated by said non-cytopathic, temperature-sensitive ~~alphaviral~~ Sindbis virus replicase.

137. (Currently amended) The DNA molecule of claim 75, wherein the non-cytopathicity is conferred by ~~one or more mutations in the nsP2 gene of said replicase~~ a substitution of proline at position 726 of the nsP2 with serine.

138. (Previously presented) The DNA molecule of claim 75, wherein temperature sensitivity is conferred by one or more mutations in the nsP4 gene of said replicase.

139. (Currently amended) The DNA molecule of claim 75, wherein the non-cytopathicity is conferred by ~~one or more mutations in the nsP2 gene of said replicase~~ a substitution of proline at position 726 of the nsP2 with serine, and temperature sensitivity is conferred by one or more mutations in the nsP4 gene of said replicase.

140. (Currently amended) The DNA vector system of claim 103, wherein the non-cytopathicity is conferred by ~~one or more mutations in the nsP2 gene of said replicase~~ a substitution of proline at position 726 of the nsP2 with serine.

141. (Previously presented) The DNA vector system of claim 103, wherein temperature sensitivity is conferred by one or more mutations in the nsP4 gene of said replicase.

142. (Currently amended) The DNA vector system of claim 103, wherein the non-cytopathicity is conferred by ~~one or more mutations in the nsP2 gene of said replicase~~ a

substitution of proline at position 726 of the nsP2 with serine, and temperature sensitivity is conferred by one or more mutations in the nsP4 gene of said replicase.

143. (Currently amended) The composition of claim 125, wherein the non-cytopathicity is conferred by ~~one or more mutations in the nsP2 gene of said replicase~~ a substitution of proline at position 726 of the nsP2 with serine.

144. (Previously presented) The composition of claim 125, wherein temperature sensitivity is conferred by one or more mutations in the nsP4 gene of said replicase.

145. (Currently amended) The composition of claim 125, wherein the non-cytopathicity is conferred by ~~one or more mutations in the nsP2 gene of said replicase~~ a substitution of proline at position 726 of the nsP2 with serine, and temperature sensitivity is conferred by one or more mutations in the nsP4 gene of said replicase.

146. (New) A method for producing a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) introducing at least one alphaviral particle of claim 91 into said host cells *in vitro*;
- (b) culturing said host cells under conditions suitable for expression of said protein or untranslated RNA molecule; and
- (c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule contained within said alphaviral particle.